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Detection of gastro-oesophageal reflux in the neonatal unit

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**Short title:** Gastro-oesophageal reflux detection in the neonatal unit

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## **ABBREVIATIONS**

ACT Acid clearance time

BCT Bolus clearance time

CI Confidence interval

MII Multichannel intraluminal impedance

NICU Neonatal intensive care unit

## **ABSTRACT**

**Aims:** To determine whether a pH probe or multichannel intraluminal impedance (MII) more frequently detected gastro-oesophageal reflux and test the hypothesis that acid reflux was associated with lower baseline impedance.

**Methods:** A prospective study of infants in whom reflux was suspected and evaluated using combined pH and multichannel impedance. Studies were considered abnormal if the acid index was >10% or there were > 79 MII reflux events in 24 hours. The acid index was the percentage of total study time with a pH < four and the acid clearance time (ACT) the time from the pH falling below four to rising above four.

**Results:** Forty-two infants (median gestational age 31 (range 23-42) weeks) were assessed. Only nine infants (21%) had abnormal studies, seven detected by pH monitoring, one by MII monitoring and one by both techniques ( $p=0.04$ ). After correcting for gestational age and postnatal age, baseline impedance remained negatively correlated with the acid index ( $r=-0.34$ ,  $p=0.038$ ) and the maximum ACT ( $r=-0.44$ ,  $p=0.006$ )

**Conclusions:** Clinical suspicion of reflux was frequently incorrect and reflux was more frequently detected by a pH probe. The inverse relationship of acid reflux to baseline impedance suggests that mucosal disruption may result from acid reflux in this population.

**Keywords:** pH study; multichannel intraluminal impedance; baseline oesophageal impedance

## KEY NOTES

- Few infants with suspected reflux gastro-oesophageal reflux disease had abnormal studies; reflux was detected significantly more frequently by a pH probe than by multichannel intraluminal impedance (MII).
- Infants with suspected reflux should be thoroughly investigated before starting on antireflux medications.
- Acid reflux indices correlated inversely with baseline oesophageal impedance suggesting that acid reflux may play a role in mucosal integrity disruption in an NICU population.

## INTRODUCTION

Gastro-oesophageal reflux is a frequent phenomenon in infants and when associated with morbidity is called gastro-oesophageal reflux disease. Reflux appears to be common in infants being cared for on neonatal units as, in the USA 24.8% of extremely low birth weight infants were discharged on anti-reflux medication (1). This, however, may reflect over prescription of anti-reflux medication. In another study of neonatal units in the USA there was a 13-fold variation in the proportion of infants who had a diagnosis of reflux (2). This variation may reflect the non-specific symptoms ascribed to reflux which include vomiting, being unsettled, back-arching and respiratory disturbance (3). Unfortunately, anti-reflux medication is not without side-effects. In a randomised trial of a proton pump inhibitor versus placebo, the infants who received the active medication had a higher occurrence of lower respiratory tract infections (4). Accurate investigation of infants suspected of reflux is thus important. Our recent survey, however, has demonstrated there is no consistency regarding the diagnostic strategies for infants with suspected reflux in UK neonatal units (5). This may reflect that the association between symptoms and the results of pH or endoscopy studies is weak (6-8). Furthermore, a reflux event is diagnosed if the pH is less than four (9-10), yet prematurely born infants may have a gastric pH of greater than four for up to 90% of the time (11) and non-acid reflux may also cause symptoms (12). Multichannel intraluminal impedance (MII) detects all oesophageal bolus movements irrespective of changes in pH. A combination of monitoring with MII and a pH probe would seem likely to result in greater detection of reflux events, but data are conflicting. Di Fiore et al. (13) used a combination of a pH and MII probe in a hospital setting including prematurely born neonates. They reported that, although weakly acid reflux events were detected by MII only, a greater number of reflux events were detected by the pH probe than MII. In another study using the combined

technique, which included 41 neonatal intensive care unit (NICU) inpatients, reflux was diagnosed in only a minority of those in whom it was suspected because of irritability, bradycardia and desaturations (14). In contrast to the previous findings (13) reflux, however, was diagnosed more often by MII (n=6) than by a pH probe (n=2).

An advantage of using MII is that intraluminal impedance not only detects bolus movement, but also may provide a measure of mucosal integrity. Damaged mucosa has a lower baseline intraluminal impedance than healthy mucosa (15, 16). Lower baseline impedance in adults has been correlated with non-erosive oesophagitis, oesophagitis and pathological acid exposure (17) and associated with microscopic evidence of mucosal disruption and greater pain with reflux (18). Lower baseline impedance may, therefore, reflect reflux associated mucosal damage. In a study of infants who were up to six months of age, baseline impedance correlated negatively with both acid and non-acid reflux indices (15). Weakly acid reflux events in asymptomatic preterm infants on the NICU occur frequently, but the clinical significance of such events in that population remains uncertain (19). Examining the correlation between reflux measurements from pH and MII studies and baseline impedance may identify which reflux measures are of clinical relevance in causing mucosal damage. An aim of this study was to determine whether a pH probe or MII more frequently detected reflux. Whether lower baseline impedance was related to reflux in infants on a neonatal unit has not been investigated and was a further aim of this study. In addition, we tested the hypothesis that acid reflux would be associated with a lower baseline impedance and determined whether there was a stronger correlation between MII derived or pH derived acid reflux with baseline impedance.

## **METHODS**

Infants with suspected reflux were assessed as part of their routine clinical care between March 2013 and August 2015. The infants were suspected of reflux because of desaturation with feeding, unexplained apnoea, poor feeding or oral aversion, poor weight gain and an apparent life-threatening event. None were receiving anti-reflux medication prior to investigation. All the infants were studied for clinical reasons, hence informed parental consent was not required. The nature and purpose of the investigations were described to the parents prior to the study, who were given the opportunity to ask for any further information and discuss any queries they had. All infants were on full enteral feeds at the time of study with some infants having a nasogastric tube in-situ. All infants were placed in their normal sleeping position ie either prone or supine.

Each infant underwent a minimum of 20 hours of continuous oesophageal pH and MII assessment. A single use combined pH/II probe (Zin51 probe, Sandhill Scientific, Highland Ranch, Colorado, USA) was used which incorporated seven impedance bands to measure impedance across six channels, each with a width of 1.5cm. In between the distal two channels was an antimony pH sensor. Prior to each study, the pH sensor was calibrated with pH buffer solutions of pH 4.0 and pH 7.0 and an automated impedance check was performed by the Zephyr Sleuth system (Sandhill Scientific). The infant's length was measured and oesophageal length estimated according to Strobel's formula for infants over 40cm in length (20) and by a nomogram for those under 40cm (21). The probe was inserted through one of the infant's nostrils and secured at the required length. A chest radiograph was then obtained as per the unit's routine policy to determine if the pH sensor was appropriately positioned between the sixth and eighth thoracic vertebra. The position of the



probe at the nares was reassessed following completion of the study to ensure the probe had not been displaced.

Following confirmation of the probe position, recording was commenced. The Zephyr Sleuth system (Sandhill Scientific) continuously recorded and impedance data with a sampling frequency of 50 Hz. Analysis of the traces produced was performed using Bioview Analysis software (Sandhill Scientific) and by manual review of the traces. A reflux event was diagnosed using the pH probe if the oesophageal pH was less than four for more than five seconds (22). The total number of pH events per 24 hours was calculated. The acid clearance time (ACT), the time from the pH falling below four to rising above four, was determined and the maximum ACT identified. The mean ACT, the total duration during which the pH was less than four divided by the number of acid reflux events was calculated. The acid index was the total time when the oesophageal pH was less than four calculated as a percentage of the total study time. Reflux was diagnosed if the acid index was greater than 10% (23).

The baseline impedance was calculated from the most distal channel using an algorithm with the effect of gas and liquid boluses being excluded (24). Each channel trace was divided into 10-minute sections which were then subdivided into 10 second epochs. The minimum impedance value for each 10 second epoch was identified. The 60 data points collected for the 10-minute section were then examined, those more than one standard deviation from the section mean were excluded as they were likely to be due to periods of bolus transit (24). The mean for the remaining points in the 10-minute period was calculated and the median for the whole study determined (24). MII reflux events were diagnosed when there was a drop-in impedance to less than 50% of the baseline at the most distal channel, which moved retrogradely across at least two channels. These were further classified as acid ( $\text{pH} < 4$ ),

weakly acid ( $4 < \text{pH} < 7$ ) or alkali ( $\text{pH} > 7$ ). The duration of a reflux event (the bolus clearance time (BCT)), was the time from the drop-in impedance to less than 50% of baseline to the time when the impedance rose above that threshold. The mean BCT was calculated and the maximum BCT noted. Reflux was diagnosed if the number of impedance detected events was greater than 79, which was the ninetieth centile in a study of forty-six healthy infants investigated for possible reflux related symptoms, who had no symptom association and an acid index less than 50% of the upper limit of normal derived from a previous study (25). In addition, this cut-off was similar to the seventy-fifth centile obtained in a study of 20 asymptomatic, prematurely born infants (19). To assess the reproducibility of scoring of the MII outputs, ten randomly selected studies were independently scored by two researchers and the reliability assessed by calculating the intra-class correlation.

## **Analysis**

Four groups were defined and analysed according to the results of the pH and MII monitoring: group 1 – negative pH and negative MII; group 2-positive pH, negative MII; group 3 – negative pH, positive MII; group 4 – positive pH and positive MII. Data were assessed for normality using histograms and the Shapiro-Wilks test. As the data were not normally distributed, differences between groups were assessed for statistical significance using the Kruskal-Wallis test. Differences between pH probe and MII detected reflux were assessed for statistical significance using the Chi square test. The strength of correlations between baseline impedance and reflux indices were determined by calculating Spearman correlation coefficients and were adjusted for postnatal and gestational age using partial rank correlations. The statistical analysis was performed using SPSS 22 (SPSS Inc, Chicago, IL).

## RESULTS

Forty-two infants (20 male) with a median gestational age of 31 (range 23-42) weeks and median birth weight of 1740 (range 550-3890) grams were assessed at a median post menstrual age (PMA) of 38 (range 30-60) weeks and postnatal age of 54 (range 2 -250) days. The infants were suspected of reflux because of desaturation with feeding (n=27), unexplained apnoea (n=6), poor feeding or oral aversion (n=3), poor weight gain (n=4) and an apparent life-threatening event (n=2). A total of 1006 hours of combined pH and MII monitoring was undertaken with a median study duration of 24 hours (range 19 -33 hours). There were 1,717 pH events with a median acid index of 4.7 (range 0-28) % and a median mean acid clearance time of 108 (range 15-318) seconds; only 585 of the events were detected by MII monitoring. MII detected 2041 reflux events; 585 (29%) were acid and detected by pH monitoring; 1387 (68%) were weakly acid and 69 (3%) alkali events. The reproducibility of MII scoring was good, as indicated by an intra-class correlation coefficient of 0.985 (95% Confidence Intervals (CI), 0.899-0.997). The median mean bolus clearance time was 18 (range 6-62) seconds and the median maximum bolus clearance time was 118 (range 16-1200) seconds.

A median of 30 (range 0-137) acid reflux events /24 hours were detected by pH studies compared to median of 13 (0-49) acid events detected by MII ( $p<0.001$ ). Furthermore, the mean duration of acid reflux events was significantly greater when derived from the pH studies compared to MII (median 108 (range 15-318) seconds versus 18 (6-61) seconds ( $p<0.001$ )).

Seven infants had an abnormal pH study only; one had an abnormal MII study only and one had both an abnormal pH and MII study. Reflux was diagnosed in more infants by the pH probe than by MII ( $p=0.04$ ). There were no significant differences in the gestational age or postnatal age between the groups (Table 1).

There was no significant difference in the baseline impedance of infants with normal or abnormal study results ( $p=0.76$ ). (Table 1). There was an inverse relationship between the baseline impedance and the maximum acid clearance time ( $r=-0.45$ ,  $p=0.003$ ), acid index ( $r=-0.37$ ,  $p=0.021$ ) and total number of pH detected events /24 hours ( $r=-0.33$ ,  $p=0.039$ ). There was a positive correlation between postnatal age and baseline impedance ( $r=0.75$ ,  $p<0.001$ ) and a negative correlation between baseline impedance and gestational age ( $r=-0.54$ ,  $p<0.001$ ). The number of pH events but not the number of MII detected events correlated negatively with corrected gestational age; baseline impedance correlated positively with corrected gestational age (Table 2). After correcting for gestational age and postnatal age, baseline impedance remained significantly inversely correlated with the maximum acid clearance time ( $r=-0.44$ ,  $p=0.006$ ) and acid index ( $r=-0.34$ ,  $p=0.038$ ). After controlling for gestational age and postnatal age, baseline impedance positively correlated with number and duration of MII detected weakly acid reflux events ( $r=0.33$ ,  $p=0.045$  and  $r=0.32$ ,  $p=0.043$  respectively). The number and duration of MII detected acid reflux events did not significantly correlate with baseline impedance either with ( $r=-.190$ ,  $p=0.253$  and  $r=-0.183$ ,  $p=0.271$ ) or without controlling for postnatal and gestational age (data not shown). Baseline impedance did not differ significantly between infants with different clinical reasons for suspecting reflux ( $p=0.29$ ) (Table 3).

## DISCUSSION

We have demonstrated using combined pH/MII monitoring in a NICU setting, that despite all the infants having a clinical suspicion of reflux, only 9 of the 42 studies (21%) were abnormal. The detection rate was higher than in the study reported by Funderburk et al (14) (14) which was only 10% of NICU patients, but lower than in a series of infants with dysphagia in whom 30% had an abnormal pH study (26). Our study importantly emphasizes a clinical suspicion of reflux is frequently incorrect and investigation should be undertaken before treatment is initiated.

The majority of infants diagnosed with reflux were detected by pH monitoring rather than by MII. Only 34% of pH detected events fulfilled the criteria for an MII reflux event in this study. This low detection rate may reflect that a reflux bolus must cross a total of three bands to be registered as a reflux event and there could be acidification of the distal oesophagus which did not propagate far enough to be registered as a reflux event. Our data then support the findings of Di Fiore (13) and Corvaglia (27), but contrast with that of Funderburk et al (14). In the latter study, however an acid index of greater than 20% was used which is much higher than in previous studies and may explain why there was a relatively low detection rate from the pH probe.

Evidence in infants of causality between reflux events and abnormal signs are limited. In a study comparing 100 infants with a clinical diagnosis of reflux to 100 healthy infants there was poor correlation between parental report of symptoms, pH study result and endoscopic evidence of oesophagitis (8). A recent study found no significant association between reflux

events and apnoea (28). Our finding of a small proportion of positive studies in those infants in whom reflux was suspected may reflect a lack of causality between reflux and reported symptoms.

The baseline impedance did not significantly differ between those infants with abnormal pH or MII studies and those with normal studies. After controlling for postnatal and gestational age, however, the maximum acid clearance time and the acid index negatively correlated with baseline impedance. In contrast, the frequency and duration of MII acid reflux events did not correlate significantly with baseline impedance suggesting they may be a poorer reflection of distal oesophageal acid exposure and mucosal disruption. Indeed, after controlling for postnatal and gestational age, the MII weakly acid reflux parameters correlated positively with baseline impedance, suggesting these reflux events may not contribute to disruption of mucosal integrity. Our results are supported by findings of a negative correlation between acid index and baseline impedance in 26 children (24). In addition, baseline impedance was demonstrated to increase following two weeks of proton pump inhibitor treatment in a randomised placebo controlled trial of 40 infants aged less than six months with symptomatic reflux (15). Our study is unique in evaluating the relationship between baseline impedance and reflux events in a NICU population. We demonstrated that our results suggest that acid reflux events may be clinically important, that is they may cause disruption of mucosal integrity. Weakly acid reflux indices did not correlate negatively with baseline impedance, suggesting that weakly acid reflux are unlikely to be contributive to oesophagitis and attempts to treat those events may not be appropriate. This is in contrast to the results from the study by Loots et al. (15) who found that baseline impedance correlated inversely with weakly acid reflux indices. The difference in the results of the two studies may be explained

by differences in the study population. Loots (15) assessed infants up to six months age in an outpatient setting whereas we assessed a neonatal unit cohort.

There are strengths and some limitations to our study. All infants underwent combined pH/MII investigation. Our sample was heterogeneous with respect to gestational and postnatal age, but reflect the population of infants cared for on a neonatal intensive care unit, thus we feel our results are generalisable. Although the infants were studied for clinical reasons, standardised assessments and referenced values to diagnose reflux were used (19, 22, 25). The number of infants with positive results were small, which limits the comparison between pH and MII results. Nevertheless, in this sample we saw a significant difference between the two techniques. In this study, we have evaluated baseline intraluminal impedance as a measure of mucosal integrity. Baseline intraluminal impedance measurement requires contact of the measurement channels with the mucosal epithelium. Absence of significant liquid bridging the impedance channels potentially reduces the accuracy of this technique in evaluating mucosal integrity. Nonetheless, intraluminal impedance measurement in rabbits has been demonstrated to show a strong correlation with transepithelial resistance measured in-vitro (29) and offers a minimally invasive means to evaluate mucosal integrity.

In conclusion, a clinical diagnosis of reflux was frequently inaccurate and reflux was more frequently detected by a pH probe than MII. Acid reflux events correlated with lower baseline impedance and pH study derived results of acid reflux correlated more strongly with baseline impedance than MII derived results of acid reflux. Nevertheless, MII derived baseline impedance results may provide an assessment of mucosal damage and indicate those infants in whom targeted acid suppression therapy may be beneficial.

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Table 1: Characteristics by investigation results. Infants grouped according to whether investigations by pH or MII study were positive or negative.

Data are presented as median (range). p-value reports comparison made across groups using the Kruskal-Wallis test.

	pH – MII – n=33	pH + MII – n=7	pH – MII + n=1	pH + MII + n=1	p-value
Gestational age (weeks)	29 (23-42)	32 (27-39)	36	32	0.61
Postnatal age (days)	56 (2-250)	23 (4-73)	18	31	0.48
Baseline oesophageal impedance( $\Omega$ )	1540 (300-2930)	1510 (620-2000)	1720	1290	0.71

Table 2: Correlation between pH/MII study results and corrected gestational age of the infant.

Data presented are the Spearman Correlation coefficient and statistical significance (p-value) of the correlation

	Correlation coefficient	Significance (p)
pH events / 24 hours	-0.31	0.046
Acid index (%)	-0.244	0.12
MII events /24 hours	-0.008	0.96
MII bolus exposure mins/24hrs	0.162	0.305
Baseline oesophageal impedance( $\Omega$ )	0.323	0.037

Table 3: Comparison of baseline impedance between groups defined by the clinical reason for suspecting reflux.

Data presented as median (range)

<b>Indication for study</b>	<b>n</b>	<b>Baseline impedance</b>
Desaturation	27	1564 (303-2928)
Apnoea	6	1325 (621-1755)
Poor feeding or oral aversion	3	1384 (1033-1486)
Poor weight gain	4	1777 (1291-2155)
ALTE	2	1381 (766-1995)